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Exhaustive extraction of the opium with lime-water in the assay procedure

In continuation of the international programme of scientific research on opium, undertaken pursuant to resolution 159 II C (VII) and 246 F (IX), adopted by the Economic and Social Council on 3 August 1948 and 6 July 1949 respectively, the Secretary-General presents the following paper prepared by chemists of the United Nations Secretariat. The question of exhaustive extraction in comparison to a single extraction of the opium with lime-water is an important one for most methods of opium assay. It is taken up in this paper with particular reference to the method explained in ST/SOA/SER.K/1, the assay method used by the United Nations Secretariat.

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EXHAUSTIVE EXTRACTION OF THE OPIUM WITH LIME-WATER IN THE ASSAY PROCEDURE

Objectives of the paper

1. The objectives of this paper are the following:
   (a) to show that exhaustive extraction of the opium may be used in the first stage of the Secretariat's method of assay, ensuring solution of the morphine and avoiding the taking of an aliquot;
   (b) to explain the advantages and disadvantages of exhaustive extraction of the opium;
   (c) to show the conclusions that may be drawn from the experiments of the Secretariat with exhaustive extraction.

Discussion

2. At the time the method of opium assay given in ST/SOA/SER.K/1 was worked out two questions relative to Stage I were temporarily postponed: the questions of complete solution of the morphine of the opium, and the exactness of the aliquot. A review of the assay problem will make clear the reasons for this postponement and the role of exhaustive extraction in Stage I.

3. The pharmacopoeial methods of opium assay, and some of the other current methods, use a lime-water solution of the opium extractives as the basis of the morphine determination. That, however, is about as far as agreement goes. Certainly, the results are widely different.

4. For example, Smeets (Die Morphinebepaling in Opium, Thesis, Rijks Universiteit, Utrecht, 1949) showed that with six different methods of opium assay, each of which has won considerable acceptance, the results on "Opium IV" ranged from 14.9 to 17.1 per cent, a difference of more than 2 per cent of the opium weight, and the results on "Opium III" ranged from 4.6 to 7.8 per cent, a difference of more than 3 per cent of the opium weight! Lesser but still serious discrepancies are quite common.

5. One major cause of such differences, occurring even with accepted pharmacopoeial precipitation methods, is the large arbitrary "correction" for morphine solubility, amounting to more than 1 per cent of the opium weight, which is added in some of these methods but not in others.

6. Is such a "correction" justified? The question comes down to that of the true amount of morphine in the lime-water solution of opium extractives. Solvent-extraction methods available prior to the United Nations method had never completely settled this, because of doubt as to the amount and effect of the minor phenolic alkaloids, which had been supposed to be inseparable from the morphine by extraction with solvents.

7. The method of ST/SOA/SER.K/1 was offered primarily as a definitive method for determining the amount of morphine in a lime-water extract of opium. This was, in fact, explained in the original discussion of the method (ST/SOA/SER.K/1, paragraph 9). The basis of an exact comparison with the official pharmacopoeial methods, or any other method using a lime-water extract of the opium, was thereby laid. For, as part of the comparison, two identical lime-water solutions can be prepared by the pharmacopoeial (or other) method. One solution can then be processed according to the pharmacopoeial method and the other extracted by the method of ST/SOA/SER.K/1.

8. However, in order to present a complete assay, the Secretariat desired to include a procedure for "Stage I", that is, for obtaining a lime-water solution of opium extractives which would represent some definite quantity of opium.

9. The problem of ensuring that all the morphine is brought into solution, although quite important, is not the cause of the most serious discrepancies between the results by different methods of assay. This is evident from the fact that two of the best-accepted methods, the International method and the method of the British Pharmacopoeia, which are among those yielding the highest results, employ only a single extraction of the opium. On the other hand the method of the United States Pharmacopoeia employs exhaustive extraction, before taking an aliquot, and yet it is among the methods yielding the lowest results.

10. The Secretariat chemists therefore concluded that a single extraction, if carefully made, should be satisfactory. This is especially true, since the morphine of a given quantity of opium is dissolved by a larger volume of solution in the Secretariat method than in pharmacopoeial methods which also use a single lime-water extraction.

11. As regards the aliquot, a great deal of attention has been given to the question of making it theoretically correct on the assumption that the morphine is all in solution; and yet this has even less bearing on the major discrepancies

between results by different methods than the question of completely dissolving the morphine. The questions of the proper solubility correction and its variation with different types of opium, in precipitation methods, and of the minor phenolic alkaloids in the older solvent-extraction methods, far overshadow the question of a theoretically perfect aliquot, which can have only a very minor effect in determinations on the usual raw opium.

12. The method of the United States Pharmacopoeia and the International method both go to great pains to make the aliquot theoretically correct, or nearly so (assuming complete solution of the morphine); but they do not agree at all in results, nor is there any real evidence that the over-all accuracy of either is superior to that of the British Pharmacopoeia method, which uses simply an approximate volume aliquot.

13. The Secretariat chemists concluded, therefore, that a simple volume aliquot, modelled on that of the British Pharmacopoeia, would be most satisfactory for the time being. Extra trouble to determine an exact aliquot is not justified as long as any increased accuracy thus gained is completely swallowed up by larger uncertainties.

14. The United Nations Secretariat method, like the methods of the British Pharmacopoeia and the United States Pharmacopoeia, determines the morphine in the opium "as is", and a calculation of the morphine content of anhydrous opium, or any reliable comparison with the results of others on opiums of varying moisture content, requires a moisture determination. The determinations made by the Secretariat on opiums of different origins have been made on "air-dry" samples. This further limited any possible variations of the aliquot, and made approximate comparisons feasible without the moisture determinations. There was, however, no intention of setting up "air-dry" opium as an exact standard. The moisture content as determined by the International method is about 5 to 7 per cent in the "air-dry" samples in this laboratory, and of course any fairly precise comparison with the results of others still requires moisture determinations.

Exhaustive Extraction in Stage I

15. Eder and Wäckerlin showed that a very considerable error may be made in the morphine determination - ranging from 0.77 to 1.37 per cent of the opium weight in their experiments - by assuming that all the morphine had been brought into solution by a single treatment with lime-water. ("The determination of Morphine in Opium", Quarterly Journal of Pharmacy and Pharmacology, 10, 680-730, 1937).

16. The Secretariat experiments have been more favorable to the single extraction than this, as will be shown. Nevertheless an incomplete single extraction must rank as one of the major possibilities of error and perhaps the chief reason that inexperienced analysts so often get too low results, regardless of the type of method used in later stages. At the best, there is still an uncertainty about a "one-shot" extraction that only an exhaustive extraction can avoid.

17. The ideal exhaustive method would provide some simple test for completion of the extraction; however, this does not seem to be practicable at present, in using lime-water extraction. The best alternative is to use a procedure that can be depended on to exhaust all the morphine from the opium, regardless of its percentage or the type of opium.

18. Eder and Wäckerlin employed exhaustive extraction; and to ensure complete solution of the morphine, they used 52 cc lime-water in seven portions for 1 gramme of opium, or 250 cc in seven portions for 5 grammes of opium. The experiments of the Secretariat indicate a very similar ration of volume as necessary to exhaust the opium. Some other methods have prescribed fewer treatments and a smaller total volume in proportion to the weight of opium taken, but such procedures may leave a doubt whether the morphine is completely extracted.

19. Thus the most important advantage of exhaustive extraction is ensuring complete solution of the morphine. This is especially valuable to the inexperienced analyst, even though the single extraction may be satisfactory in the hands of those skilled in opium analysis.

20. In the International method moisture and extract determinations are made in addition to the morphine precipitation, in order to calculate a theoretically exact aliquot. But uncertainty remains, because only a single extraction is made.

21. Exhaustive extraction is more certain, and yet avoids the necessity of moisture and extract determinations, which may not be needed otherwise. It likewise avoids any possible inaccuracy of an approximate volume aliquot. This is of value in extending the method to forms of opium for which the specified volume aliquot might be inappropriate, e.g., smoking opium with a high content of moisture.

22. Therefore, although it is quite possible to use the single lime-water extraction and an approximate volume aliquot and still obtain a good analysis on the usual raw opium, it is better to use exhaustive extraction if it does not cost too much in time and trouble.

23. In short, exhaustive extraction has every advantage over the single extraction except that it entails more work and yields a much greater quantity of solution for a given weight of opium. In the United Nations Secretariat method the greater volume of solution means the use for the first solvent extraction, of at least two continuous extractors, or better three, instead of one - if the extractors are of the size previously used, and if the extract is still to represent 4 grammes of opium (see E/CN.7/195 for a diagram of the solvent extraction apparatus used). The solvent extracts can be combined for evaporation, and thereafter the procedure can be the same as for the single extraction with lime-water.

24. In the method of the United States Pharmacopoeia an exhaustive extraction of the opium is made with plain water, and the extract is then evaporated down to a comparatively small volume and converted to a lime-water solution, from which an aliquot is taken. This is necessary in order to have only a small volume for precipitation; but this method also involves considerable time and trouble, and possibly some elements of uncertainty. It might be used to reduce the volume if only a single continuous extractor is available.

25. In view of the preceding "Discussion", paragraphs 2 to 14, it should now be shown that the exhaustive extraction is worth the extra trouble: that the method of the Secretariat, as a whole, is such that the gain in certainty in Stage I is not thrown away or completely overshadowed by more serious uncertainties later on. Otherwise the shorter and faster procedure of a simple volume aliquot would still be preferable for Stage I.

26. The method of the United Nations Secretariat eliminates any question of the amount of morphine normally remaining in solution, and any question of codeine seriously affecting the result; questions which affect the pharmacopoeial precipitation methods. It also eliminates at least the known error, due to minor phenolic alkaloids, of previous extraction methods. Studies are being pursued to determine whether any positive error, due to the inclusion of some unknown titratable substance, with the morphine, is still possible. If so, it has not been established, and cannot be large. It seems, therefore, that the greatest remaining uncertainty is, not the aliquot as such, but the complete solution of the morphine in the lime-water. Therefore the exhaustive extraction is advisable for a rigorous determination in spite of the added time and trouble.

27. Some studies are still under way to determine the best combination of solvents for the extraction of Stage II. In the following method, benzene-isopropanol is directed for the first extractor and benzene-ether-acetone for the second. However, either combintation could be used for both extractions. Benzene-isopropanol is advantageous for speed, while benzene-ether-acetone yields a remarkably "clean" extraction.

Method

28. Weigh out 4.000 gm of the opium into a large mortar. Add 15 cc water and 2 gm Ca $(OH)_2$, grind up and rub to a smooth paste, then add 50 cc more water and continue rubbing and stirring during about 30 minutes. In the meantime put 1 gm more Ca $(OH)_2$ in a flask with about 200 cc water and shake occasionally. Use this lime-water for subsequent extractions.

29. Filter the opium solution with suction, preferably using a Buchner-type sintered-glass filter. (If faucet suction is used, use a trap to be sure there is no back-flow of water into the filtrate.) Suck the residue as dry as possible. Pour this filtrate into a 100 cc graduate.

30. Scrape the opium back into the mortar with a glass rod, and add enough lime-water (about 25-30 cc) to make up the difference between the volume of filtrate already in the graduate and 70 to 75 cc. Rework for about 15 minutes, then filter with suction as before, and pour the filtrate into the graduate.

31. Charge a continuous extractor with the filtrate, NH_4Cl, and 200 cc of benzene-isopropanol (150 : 50). Use an amount of NH_4Cl as follows: for 70 cc lime-water solution, 2 gm; for 75 cc, 2.15 gm; for 80 cc, 2.3 gm NH_4Cl. In charging the extractor, to prevent the aqueous liquid from running through the porous plate, pour in the aqueous solution and part of the solvent solution at the same time. Then connect the apparatus and pour the remaining solvent in through the condenser. Begin extraction at once. Continue until the extract from a small portion of the returning solvent, evaporated in a small porcelain crucible, no longer shows an indication of morphine with Fröhde's reagent (0.5 gm sodium or ammonium molybdate in 100 cc concentrated H_2SO_4).

32. With the suction cut off, rinse any opium marc remaining in the mortar onto the filter with about 20 cc lime-water and work up the opium marc thoroughly on the filter; then apply the suction. Pour the filtrate into the graduate. Rework

the opium marc on the filter, with the suction cut off, three times more, with about 20 cc lime-water each time, collecting a total of about 75 cc lime-water extract.

33. Charge a second continuous extraction apparatus with the filtrate sufficient NH_4Cl (paragraph 31), and 100 cc of ether-acetone (60 : 40). As soon as the extraction apparatus is charged with the opium solution, connect it and pour any remaining ether-acetone in through the condenser. Also pour in through the condenser 75 cc of benzene-acetone (45 : 30). Stand the extraction flask in a beaker containing a little water on a hot plate, and extract as usual. Test for completion of the extraction with Maquis or Frõhde's reagent.

34. Return the opium marc to the mortar and rework with 25 cc lime-water. Filter. Rework twice more on the filter, with the suction turned off, with about 25 cc water each time, to collect a total of about 75 cc more lime-water extract. Put in a third continuous extractor with NH_4Cl and 150 cc of ether-acetone (90 : 60). Extract as usual, testing for completion with Frõhde's reagent.

35. The solvent extracts from the first two extractors, or from all three, can be combined during evaporation to yield a single residue, which is then processed for morphine as usual. In the Secretariat experiments the extract from the third extractor was processed separately, in attempting to work out the method so as to require only two extractors. It may still be advisable to process it separately for a few times, while an analyst is inexperienced, as a check on the technique.

36. In Stage III, if the residue from the third extractor is processed separately, it is sufficient to use two acid solutions. Besides 25 cc chloroform, 0.3 cc dichloracetic acid and 10 cc water are used in transferring the residue to the first separatory. The second separatory contains 5 cc water, with no dichloracetic acid added directly (the chloroform carries some along). Extract the other alkaloids with additional portions of 25-15 cc $CHCl_3$. In Stage IV omit the first 50 cc of solvent mixture and begin extracting with 25 cc of chloroform-isolutanol (75 : 25). Test a little of the third or fourth such extract to see if the morphine is already all out. Continue the extraction, if necessary, with 20 : 5 chloroform-isobutanol. In Stage V titrate this residue with n/50 or n/100 acid.

The Single Extraction

37. Weigh out 6.000 gm of opium (preferably, but not necessarily, in the "air-dry" condition); put into a large mortar with 20 cc. water and 3 gm $Ca(OH)_2$, and grind to a uniform paste. Add 70 cc. water and stir thoroughly and very frequently for 30 minutes, carefully rubbing the opium marc to make disintegration as complete as possible. Filter and take 61 cc of the filtrate to represent 4 gm of opium. (This aliquot, which includes an allowance of 1 cc for extractives, is justified by the results given below).

38. In the International method, the lime is added with the first water (as in the preceding paragraph), and the mixture worked up. In the method of the British Pharmacopoeia, the opium is first worked up and thoroughly disintegrated with water alone, before the lime is added. This sometimes makes the process of disintegration more difficult, and the Secretariat chemists have not found convincing evidence that such a precaution is of much help in ensuring complete solution of the morphine. They have found, however, that it is inadvisable to grind the opium and lime together before adding water.

39. In both the British Pharmacopoeia and the International methods, treatment by grinding is followed by rinsing into a flask, and shaking. The Secretariat chemists have not found this necessary, and it seems very doubtful if shaking is any more likely, or even as likely to ensure complete solution of the morphine as continued stirring in the mortar, with rubbing of any opium marc that resists disintegration. On the other hand, the use of a Blendor may sometimes be advantageous, though this has not been rigorously tested. The Blendor produces some foam, but filtration with suction will yield the specified aliquot.

40. Another requisite for successful use of the single extraction is that the lime be full strength. In spite of the fact that the lime is used in considerable excess, this alone is not sufficient, and some analysts prefer to use their own recently slaked lime. However, the commercial $Ca(OH)_2$, which has been well protected from the atmosphere, should be satisfactory. The International method includes a test for the strength of the lime.

Results and observations

41. Although the results by the method of the United Nations Secretariat are generally among the highest, in the case of the old League of Nations samples they were lower than expected from the original analyses of about 1935-1937, on samples III, IV, and V. (Iran, Turkey, and Yugoslavia). There have previously been reports of loss of morphine in powdered opium during storage, and it seems probable that it has occurred in this case. (See, especially, Annett and Singh, "The loss of morphine content of powdered opium on storage", Pharmaceutical Journal 109, 304-305, 1922.) This point will be studied further.

42. Comparisons between the results by exhaustive extraction and satisfactory determinations by single extraction, are as follows:

Sample	Morphine by Single extraction	Exhaustive extraction
L.of N. I. Benares (India)	10.55,10.47	10.66
L.of N. II. Calcutta (India)	11.04	11.18
L.of N. III. Iran	10.57	10.51,10.40
L.of N. IV. Turkey	10.55	10.60
L.of N. V. Yugoslavia	15.29,14.94	15.18
India No.3 - Indore State	9.52	9.56
U.N. 6 - Turkey - Nallihan-B	13.45	13.26
U.N. 49 - Iran - Kerman	11.65,11.81	11.56

43. As far as small discrepancies in these results are concerned, it should be remembered that a difference of 0.1 - 0.2 means a difference of only two or three drops of tenth-normal acid in the titration, at the end of a long and complex separation. The hundredth place is really quite uncertain, but is allowed to stand here to show the comparison of results more clearly.

44. It is only too easy for some accident or error to make a result definitely too low. In the experiments of the Secretariat a low result has occurred a number of times when it has seemed that the deficiency must be due to incomplete solution in the single extraction method. In such cases the results have been low by amounts ranging up to as much as 1.6 per cent of the opium weight. In other words, deficiencies of the order found by Eder and Wäckerlin, or nearly as

great, may occur rather easily when only a single extraction is used, even with the greater amount of lime-water in the method of ST/SOA/SER.K/1 as compared to most precipitation methods. However, the figures show that complete solution can be obtained with the single extraction, on a wide variety of opium types.

45. In the Secretariat's determinations the residue from the third extractor (solvent extraction of Stage II) was processed separately. The amount of morphine found in it was, roughly, about one-half per cent of the total amount; the maximum found was 0.14 per cent of the opium weight, the average was 0.08 per cent of the opium weight. Despite efforts to get all the morphine into the first two extractors, for example by increasing the number of separate treatments of the opium with lime-water to obtain the total volume of about 150 cc., qualitative tests always showed a little morphine obtained from the third extractor, with a titration corresponding to at least 0.03 or 0.04 per cent of the opium weight. In two or three cases a re-extraction was necessary to obtain the titration. It was ascertained that microcrystal identification tests for morphine, as well as the characteristic colour tests, could be obtained on the final residue from the third extractor.

Conclusions

46. A single extraction of opium with lime-water (as in the International and British Pharmacopoeia methods of assay) is capable of giving satisfactory results. However it is difficult to obtain complete solution of the morphine with a single extraction, and almost impossible to be perfectly sure of it.

47. If single extraction is used, the accuracy of a simple volume aliquot (as in the method of the British Pharmacopoeia) is satisfactory for ordinary raw opium. Any uncertainty thus introduced is far overshadowed by others, including the uncertainty of the single extraction itself.

48. Exhaustive extraction is far more certain. It is certainly advisable for a rigorous determination, but is fully justified only when other large uncertainties are eliminated, as in the method of the United Nations Secretariat.

49. Exhaustive extraction requires a minimum of approximately 50 cc lime-water per gramme of opium, in about seven separate treatments. Since complete solution of the morphine is possible with a single extraction, the difficulty of getting it out appears to lie largely in the opium holding the solution like a sponge.

50. In the Secretariat's experiments the very small amount of morphine recovered from the third extractor shows two things: (a) that assuredly all the morphine obtainable by lime-water was exhaustively extracted from the opium; (b) that no titratable substance, capable of staying with the morphine in the Secretariat's method and soluble in line-water but less so than morphine, exists in opium in any considerable quantity - otherwise it would certainly be noted in the final residue from the extracts processed in the third extractor.

51. With exhaustive extraction of the opium in Stage I, the method of the United Nations Secretariat, as far as can be ascertained at present, has all the elements of a rigorous determination of the morphine content of opium.